CLAIMS

1. A method of creating a library of artificial promoters comprising

a) obtaining an insertion DNA cassette, which comprises, a first recombinase site, a second recombinase site and a selective marker gene located between the first and the second recombinase sites;

b) obtaining a first oligonucleotide which comprises, i) a first nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest, and ii) a second nucleic acid fragment homologous to a 5' end of the insertion DNA cassette;

c) obtaining a second oligonucleotide which comprises, i) a third nucleic acid fragment homologous to a 3' end of said insertion DNA cassette, ii) a precursor promoter comprising a -35 consensus region (-35 to -30), a linker sequence and a - 10 consensus region (-12 to - 7), wherein the linker sequence comprises between 14 - 20 nucleotides and is flanked by the -35 region and the -10 region, wherein said precursor promoter has been modified to include at least one modified nucleotide position of the precursor promoter and wherein the -35 region and the -10 region each include between 4 to 6 conserved nucleotides of the promoter, and iii) a fourth nucleic acid fragment homologous to a downstream region of the transcription start site of the promoter; and

d) mixing the first oligonucleotide and the second oligonucleotide in an amplification reaction with the insertion DNA cassette to obtain a library of double stranded amplified products comprising artificial promoters.

2. The method according to claim 1 further comprising purifying the amplified products.

3. The method according to claim 1, wherein the amplification step is a polymerase chain reaction step.

4. The method according to claim 1, wherein the -35 region of the precursor promoter is selected from the group consisting of TTGACA, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA and TTCAAA.

5. The method according to claim 1, wherein the -35 region of the precursor promoter comprises a modification to the -30 residue of the precursor promoter.

6. The method according to claim 1, wherein the -10 region of the precursor promoter is selected from the group consisting of TAAGAT, TATAAT, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT.

7. The method according to claim 1, wherein the -35 region of the precursor promoter is TTGACA and the -10 region of the precursor promoter is TATAAT.

8. The method according to claim 1, wherein the 35 region of the precursor promoter is TTGACA and the -10 region of the precursor is AATAAT.

9. The method according to claim 1, wherein the linker sequence comprises between 16 and 18 nucleotides.

10. The method according to claim 1, wherein the precursor promoter is obtained from a promoter selected from the group consisting of P_{trc} (SEQ ID NO 2); $P_{D/E20}$ ((SEQ ID NO. 4); P_{H207} (SEQ ID NO. 3); P_{N25} (SEQ ID NO. 5); P_{G25} (SEQ ID NO.6); P_{J5} (SEQ ID NO.7); P_{A1} (SEQ ID NO. 8); P_{A2} (SEQ ID NO. 9); P_{A3} (SEQ ID NO. 10); P_{lac} (SEQ ID NO. 1); P_{GI} (SEQ ID NO. 15); P_{lacUV5} (SEQ ID NO. 12); P_{CON} (SEQ ID NO.4); and P_{bls}(SEQ ID NO. 14).

11. The method according to claim 1, wherein the library of artificial promoters includes SEQ ID NO. 15, SEQ ID NO. 16 and SEQ ID NO. 17.

12. The method according to claim 1, wherein the precursor promoter and the chromosomal gene of interest are heterologous.

13. The method according to claim 1, wherein the precursor promoter and the chromosomal gene of interest are homologous.

14. The method according to claim 1 further comprising modifing the ribosome binding site including,

d) obtaining a third oligonucleotide which comprises,

i) a fifth nucleic acid fragment homologous to the 5' end of said chromosomal gene of interest,

ii) a modified ribosome binding site of the gene of interest, said ribosome binding site includes at least one modified nucleotide, and

iii) a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and

e) mixing the PCR products of claim 1 with the third oligonucleotide and the first oligonucleitde of claim 1 in a PCR reaction to obtain PCR products comprising artificial promoters with modified ribosome binding sites.

15. The method according to claim 14, wherein the ribosome binding site from the precursor promoter is selected from the group consisting of AGGAAA, (SEQ ID NO. 30), AGAAAA

(SEQ ID NO. 31), AGAAGA (SEQ ID NO. 32), AGGAGA (SEQ ID NO. 33), AAGAAGGAAA (SEQ ID NO. 34), AAGGAAAA (SEQ ID NO. 35), AAGGAAAG (SEQ ID NO. 36), AAGGAAAU (SEQ ID NO. 37), AAGGAAAAA (SEQ ID NO. 38), AAGGAAAAG (SEQ ID NO. 39), AAGGAAAAU (SEQ ID NO. 40), AAGGAAAAAA (SEQ ID NO. 41), AAGGAAAAAG (SEQ ID NO. 42), AAGGAAAAAU (SEQ ID NO. 43), AAGGAAAAAAA (SEQ ID NO. 44), AAGGAAAAAAG (SEQ ID NO. 45), AAGGAAAAAAU (SEQ ID NO. 46), AAGGAAAAAAAA (SEQ ID NO. 47), AAGGAAAAAAAG (SEQ ID NO. 48), AAGGAAAAAAAU (SEQ ID NO. 49), AAGGAAAAAAAAA (SEQ ID NO. 50), AAGGAAAAAAAAG (SEQ ID NO. 51), AAGGAAAAAAAAU (SEQ ID NO. 52), AAGGAAAAAAAAAA (SEQ ID NO. 53), AAGGAAAAAAAAAG (SEQ ID NO. 54), AAGGAGGAAA (SEQ ID NO. 55), and AAGGAAAAAAAAAU (SEQ ID NO. 56).

16. The method according to claim 14 further comprising inserting a stabilizing mRNA sequence between the modified ribosome binding site and a transcription initiation site of the third oligonucleotide.

17. The method of claim 14, further comprising altering the start codon of the gene of interest in the third oligonucleotide.

18. The method according to claim 1 further comprising,

d) obtaining a third oligonucleotide comprising

i) a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest in claim 1,

ii) a start codon of the gene of interest, wherein said start codon is degenerated and includes at least one modification oligonucleotide and

iii) a sixth nucleic acid fragment homologous to the downstream region of the -10 region of the second oligonucleotide , and

e) mixing the PCR products of claim 1 with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified start codons.

19. The method according to claim 17 further comprising inserting a stabilizing mRNA sequence between the -10 box of the artificial promoter and a transcription initiation site of the third oligonucleotide.

20. The artificial promoter library produced by the method of claim 1.

21. The artificial promoter library produced by the method of claim 2.

22. An artificial promoter library comprising a mixture of double stranded polynucleotides which include in sequential order:

a) a nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest,

b) a first recombinase site,

c) a nucleic acid sequence encoding an antimicrobial resistance gene,

d) a second recombinase site,

e) two consensus regions of a promoter and a linker sequence, wherein the first consensus region comprises a -35 region, the second consensus region comprises a -10 region and the linker sequence comprises at least 14 - 20 nucleotides and is flanked by the first consensus region and wherein the -35 region and the -10 region each include between 4 - 6 conserved nucleotides of corresponding consensus regions of the promoter, and

f) a nucleic acid fragment homologous to the downstream region of the +1 transcription start site of the promoter.

23. The artificial promoter library of claim 22, wherein the double stranded polynucleotides further include a modified ribosome binding site of the promoter wherein said binding site is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site.

24. The artificial promoter library of claim 22, wherein the double stranded polynucleotides further include a modified start codon, wherein the modified start codon sequence is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site.

25. The artificial promoter library of claim 22, wherein the double stranded polynucleotides further include a stabilizing mRNA nucleic acid sequence, wherein the stabilizing mRNA sequence is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site.

26. The artificial promoter library of claim 22, wherein the -35 region includes a substitution in one nucleotide position with the remaining nucleotide positions conserved.

27. The artificial promoter library of claim 26, further including a substitution in one nucleotide position of the -10 region with the remaining nucleotide positions conserved.

28. A method of modifying a promoter in selected host cells comprising

a) obtaining a library of PCR products comprising artificial promoters according to claim 1;

b) transforming bacterial host cells with the PCR library, wherein the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination;

c) growing the transformed bacteria cells;

d) selecting the transformed bacterial cells comprising the artificial promoters.

29. A method of modifying a promoter in selected host cells comprising

a) obtaining a library of PCR products comprising artificial promoters according to claim 14;

b) transforming bacterial host cells with the PCR library, wherein the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination to produce transformed bacterial cells;

c) growing the transformed bacteria cells;

d) selecting the transformed bacterial cells comprising at least one artificial promoter.

30. A method of modifying a promoter in selected host cells comprising

a) obtaining a library of PCR products comprising artificial promoters according to claim 18;

b) transforming bacterial host cells with the PCR library, wherein the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination to produce transformed bacterial cells;

c) growing the transformed bacteria cells;

d) selecting the transformed bacterial cells comprising at least one artificial promoter.

31. The method according to claim 28, wherein the bacterial host cell is selected from the group consisting of *E. coli*, Pantoea *sp.* and *Bacillus sp.*

32. The method according to claim 29, wherein the bacterial host cell is selected from the group consisting of *E. coli*, *Pantoea sp.* and *Bacillus sp.*

33. The method according to claim 30, wherein the bacterial host is selected from the group consisting of *E. coli*, *Pantoea sp.* and *Bacillus sp.*

34. A method of creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprising,

a) obtaining a library of PCR products comprising artificial promoters according to claim 1;

b) transforming bacterial host cells with the PCR products, wherein the PCR products comprising the artificial promoters are integrated into bacterial host cells by homologous recombination to produce transformed bacterial cells;

c) growing the transformed bacteria cells; and

d) obtaining a library of transformed bacterial cells wherein the library exhibits a range of expression levels of a chromosomal gene of interest.

35. The method according to claim 34, further comprising selecting transformed bacterial cells from the library.

36. The method of claim 35, wherein the selected transformed bacterial cells have a low level of expression of the gene of interest.

37. The method of claim 35, wherein the selected transformed bacterial cells have a high level of expression of the gene of interest.

38. The method according to claim 35 further comprising excising the selective marker gene from the transformed bacterial cells.

39. Transformed bacterial cells selected according to the method of claim 35.

40. The method according to claim 35, wherein the bacterial host cell is an *E. coli*, *Bacillus sp.* or *Pantoea sp.* cell.